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Pseudobarbus kubhekai sp. nov., a new redfin (Teleostei, Cyprinidae) from KwaZulu-Natal, South Africa

Fatah Zarei[1], Xiluva Mathebula[1], Albert Chakona[1,2]

1 *NRF-South African Institute for Aquatic Biodiversity (NRF-SAIAB), P. Bag 1015, Makhanda (Grahamstown) 6140, South Africa*
2 *Department of Ichthyology and Fisheries Science, Rhodes University, PO Box 94 Makhanda (Grahamstown), 6140, South Africa*

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Corresponding author: Albert Chakona (A.Chakona@saiab.nrf.ac.za)

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Abstract

A recent phylogeographic analysis of the remnant populations of *Pseudobarbus quathlambae* from Lesotho and South Africa revealed the existence of three allopatrically distributed lineages: (i) one in eastern Lesotho, (ii) one in Mohale, central Lesotho, and (iii) a third lineage in the Umzimkhulu (= Mzimkhulu) River, KwaZulu-Natal, adjacent to the Mkhomazana River (type locality), where the species has gone extinct. The present study provides morphological and osteological evidence corroborating the distinctiveness of the Umzimkhulu River population from all other populations (extant and extinct) of *P. quathlambae*, supporting their recognition as distinct species. Herein, we describe the Umzimkhulu River population as a new species, *Pseudobarbus kubhekai*. The new species, a single barbeled redfin, differs from all currently recognized congeners by having 51–56 scales in lateral-line series (vs. 60–72 in *P. quathlambae* and 25–45 in the other species). Proposed steps to resolve the taxonomic status of *P. quathlambae* from other localities (Mkhomazana population, Eastern Lesotho Highlands, and Mohale lineages) are presented.

Key Words

Cyprinidae, endemic species, freshwater fish, systematics, Umzimkhulu redfin

Introduction

The cyprinid genus *Pseudobarbus* Smith, 1841, commonly referred to as redfins, encompasses a unique group of small freshwater fishes endemic to South Africa and Lesotho (Skelton 1988, 2001). These species are distinguished by their bright red fins, which is a notable characteristic setting them apart from other cyprinids from southern Africa. Taxonomically, *Pseudobarbus* has had a relatively complex history. Initially grouped under the broader genus *Barbus* Daudin, 1805, *Pseudobarbus* was later recognized as a distinct genus based on unique morphological traits, in particular, possession of a flexible primary dorsal spine and development of prominent nuptial tubercles in breeding males, in addition to the distinctive bright red scarlet patches at the base of the fins (Skelton 1988). Molecular data subsequently supported the monophyly of this lineage (Swartz et al. 2009), with karyological data also showing that these fishes are tetraploid (Naran et al. 2006). The genus currently comprises 11 valid species, but molecular data indicates the presence of several additional lineages that await formal description (Chakona et al. 2013; Swartz et al. 2004, 2007, 2009, 2023).

Pseudobarbus quathlambae (Barnard, 1938) is the only redfin species that is not associated with the Cape Fold Ecoregion, as it is isolated in the Drakensberg-Maloti Highlands and the Southern Temperate freshwater ecoregions (Fig. 1; Skelton 1974, 1988; Kubheka et al. 2017). This geographic isolation is reflected in its phylogenetic relationships because it represents the earliest diverging lineage within the genus, and genetically, it is the most divergent *Pseudobarbus* species (Swartz et al. 2009). It has been one of the flagship species for conservation in southern Africa, particularly in Lesotho, as it is the only endemic vertebrate in this country (Skelton 2000). The species was first discovered in the Mkhomazana River

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(a tributary of the Mkhomazi/Mkomazi River system) at the foot of the Drakensberg Mountains in the KwaZulu-Natal Province of South Africa (Fig. 1; Barnard 1938; Jubb 1966), where it was reportedly abundant in 1938 (Pike and Tedder 1973). However, predation and competition from trout introduced into the Mkhomazi River between 1910–1927 caused the extinction of this population (Jubb 1966; Pike and Karssing 1995).

More recently, a redfin population was discovered in the Umzimkhulu (= Mzimkhulu) River system in South Africa, adjacent to the Mkhomazi River, where the species has gone extinct (Kubheka et al. 2017; Fig. 1). The newly discovered population was thus preliminary assigned to *P. quathlambae* (Kubheka et al. 2017). A recent phylogeographic analysis of the remnant populations of *P. quathlambae* in Lesotho and South Africa by Swartz et al. (2023) identified three allopatrically distributed lineages with deep genetic divergence (2.3–6.4%) in the sequence of mitochondrial control region: (i) one in eastern Lesotho, (ii) one in Mohale, central Lesotho, and (iii) a third lineage confined to the Umzimkhulu River system in the KwaZulu-Natal (KZN) province of South Africa. This initially raised two possibilities: (i) the new KZN population represents *P. quathlambae*, whereas (ii) the Lesotho Highlands populations represent one or two new species for science. Preliminary morphological examination of the newly discovered Umzimkhulu population, however, revealed a clear separation from examined specimens from the Lesotho highlands. Meristic counts were also distinctly different from those reported in the original description of *P. quathlambae*.

In the present study, we undertook detailed molecular (analysis of mtDNA Cyt *b* sequences), morphological, and osteological examination of the Umzimkhulu and Lesotho Highlands specimens and compared them to the original description of *P. quathlambae*. We found morphological and meristic evidence corroborating the genetic distinctiveness of the Umzimkhulu River population from all other populations of *P. quathlambae* sensu lato [i.e., the original specimens from Mkhomazi River (based on data in the original description), eastern Lesotho, and Mohale], supporting the recognition of the former as a distinct species. Herein, we describe the Umzimkhulu River population as a new species, *Pseudobarbus kubhekai*. Determination of the taxonomic status of the Mohale and eastern Lesotho highlands population will be dealt with in a future study that will attempt to generate mtDNA sequences from the preserved type specimens of *P. quathlambae*.

Materials and methods

Institutional abbreviations follow Sabaj (2010) and are listed at http: www.asih.org/node/204. The description of *P. kubhekai* sp. nov. is based on 15 specimens that were collected from the Umzimkhulu River system during a survey conducted in May 2017 (Fig. 1). The type material is deposited at the South African Institute for Aquatic Biodiversity (NRF-SAIAB).

Molecular data

Following Chakrabarty (2010), three Cyt *b* sequences of *Pseudobarbus kubhekai* sp. nov. from the Umzimkhulu

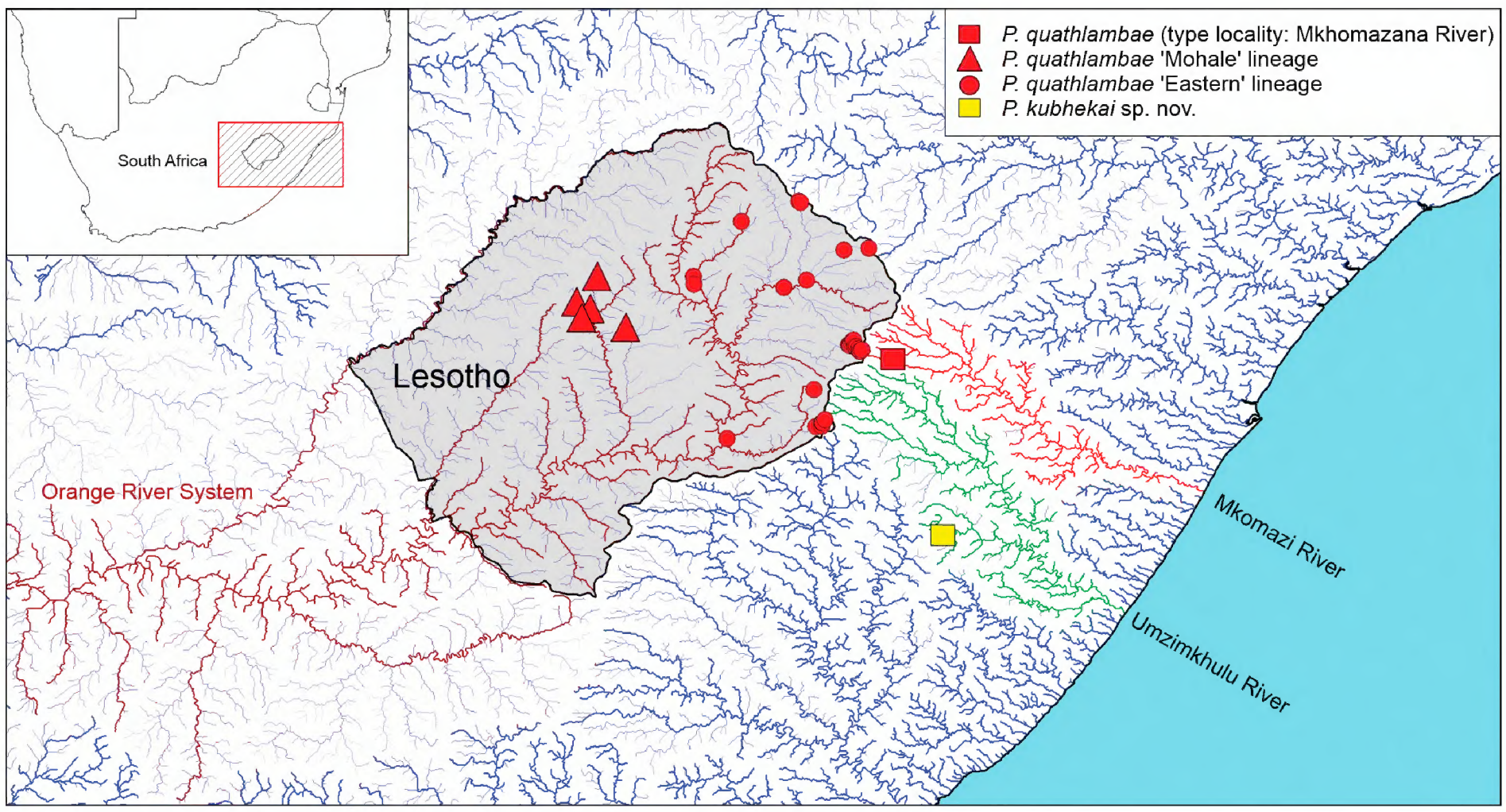

Figure 1. Distribution of *Pseudobarbus kubhekai* sp. nov. (yellow square) in the Umzimkhulu River and of *P. quathlambae* (red symbols) in the Mkomazi/Mkhomazi and upper Orange River systems. The red square shows the type locality of *P. quathlambae* in the Mkhomazana River, a tributary of the Mkomazi River system.

River were designated as hologenetypes and paragenetypes (Sequence IDs. SB12344 to SB12346; GenBank Nos. PQ367261 to PQ367263). To determine the phylogenetic placement of the new species, 18 Cyt *b* sequences of *Pseudobarbus* spp. were retrieved from Swartz (2005), Chakona and Skelton (2017), Tsigenopoulos et al. (2002), Machordom and Doadrio (2001), and Chakona et al. (2013, 2014) and included in the phylogenetic analysis (Table 1). Swartz et al. (2009) were followed for DNA extraction, amplification, and sanger sequencing of the PCR products.

The Cyt *b* sequences were first manually edited with BioEdit v. 7.2.5 (Hall 1999), then aligned using the ClustalW algorithm in Mega 7 (Kumar et al. 2016); finally, the alignment was trimmed to an equal fragment length of 665 bps. Substitution saturation in the Cyt *b* dataset was tested with DAMBE 7 (Xia 2018) using the method described by Xia et al. (2003). The substitution model best fitting the sequences (GTR+I+G) was determined using the Bayesian Information Criterion (BIC) in JModelTest 2.1.3 (Darriba et al. 2012). MrBayes 3.2.6 (Ronquist et al. 2012) was used for Bayesian inference (BI) of phylogeny (20,000,000 generations, 20% burnin). *Sedercypris calidus* (GenBank No. AF287423) was included as an outgroup. The resulting phylogenetic tree was edited in FigTree 1.4.4 (Rambaut and Drummond 2012). Average sequence divergence was estimated using the Kimura-2-Parameter (K2P) model in Mega. To delineate hypothetical species based on molecular data, three conceptually different methods of species delimitation were applied: (i) a distance-based method, the Assemble Species by Automatic Partitioning (ASAP; Puillandre et al. 2021); (ii) a network-based method, the reversed statistical parsimony (SP) in TCS 1.21 (Clement et al. 2000); and (iii) a topology-based method, the Bayesian Poisson Tree Process (bPTP; Zhang et al. 2013).

Morphological data

Meristic and morphological characters were examined following Hubbs and Lagler (1958), Skelton (1988), Chakona and Swartz (2013), and Chakona and Skelton (2017). The characters considered for each specimen in the present study include 22 morphometric measurements and 12 meristic counts. We compared morphological and meristic differences among all single barbeled redfins using raw data from Skelton (1980, 1988) and Chakona and Skelton (2017). Principal Component Analysis (PCA) was performed on raw meristic variables and morphometric variables in percentages to explore the separation of the specimens and identify the variables that contribute the most to differences among groups. Statistical analyses were performed with IBM SPSS Statistics 27 and PAST 4.16c (Hammer et al. 2001). Osteology was checked based on X-ray images prepared with an Inspex 20i Digital Radiography System (Kodex Inc., New Jersey, USA), housed at NRF-SAIAB.

Table 1. List of *Pseudobarbus* specimens used in the phylogenetic analysis (Cyt *b*), including GenBank accession numbers.

Species	Lineage	Locality	GenBank number (sequence ID)	Reference
P. kubhekai sp. nov.		South Africa: Umzimkhulu River	PQ367261 (SB12344)	This study
P. kubhekai sp. nov.		South Africa: Umzimkhulu River	PQ367262 (SB12345)	This study
P. kubhekai sp. nov.		South Africa: Umzimkhulu River	PQ367263 (SB12346)	This study
P. quathlambae (Barnard, 1938)	Mohale	Central Lesotho	AY791827	Swartz (2005)
P. quathlambae (Barnard, 1938)	Mohale	Central Lesotho	AY791833	Swartz (2005)
P. quathlambae (Barnard, 1938)	Eastern	Eastern Lesotho	AY791824	Swartz (2005)
P. quathlambae (Barnard, 1938)	Eastern	Eastern Lesotho	AY791825	Swartz (2005)
P. quathlambae (Barnard, 1938)	Eastern	Eastern Lesotho	AY791817	Swartz (2005)
P. afer (Peters, 1864)		South Africa: Sundays River	KY472280	Chakona and Skelton (2017)
P. afer (Peters, 1864)	Forest	South Africa: Klein Brak River	SB11794	NRF-SAIAB (Unpublished data)
P. swartzi Chakona & Skelton, 2017		South Africa: Gamtoos River	KY472266	Chakona and Skelton (2017)
P. senticeps (Smith, 1936)		South Africa: Krom River	KY472274	Chakona and Skelton (2017)
P. asper (Boulenger, 1911)		South Africa: Groot River	AF180850	Tsigenopoulos et al. (2002)
P. tenuis (Barnard, 1938)		South Africa: Vlei	AF287453	Machordom and Doadrio (2001)
P. phlegethon (Barnard, 1938)		South Africa: Noordhoeks	AF287452	Machordom and Doadrio (2001)
P. burchelli (Smith, 1841)	Breede	South Africa: Breede River	KF222732	Chakona et al. (2013)
P. burchelli (Smith, 1841)	Heuningnes	South Africa: Heuningnes River	KF222788	Chakona et al. (2013)
P. burchelli (Smith, 1841)	Tradou	South Africa: Tradou River	KF222702	Chakona et al. (2013)
P. skeltoni Chakona & Swartz, 2013		South Africa: Riviersonderend River	KF222586	Chakona et al. (2013)
P. burgi (Boulenger, 1911)		South Africa: Berg River	AF180849	Tsigenopoulos et al. (2002)
P. verloreni Chakona, Swartz & Skelton, 2014		South Africa: Verlorenvlei River	KM366106	Chakona et al. (2014)

Results

Molecular data

A substitution saturation test of Cyt *b* sequences showed that all codon positions are not saturated and could be used for phylogenetic analysis. The three sequences of *P. kubhekai* sp. nov. defined one haplotype belonging to a lineage sister group to a clade comprising the *P. quathlambae* 'Mohale' and *P. quathlambae* 'Eastern' lineages (Fig. 2). The range of K2P genetic distance (%) between *P. kubhekai* sp. nov. and the other *Pseudobarbus* species/lineages is 6.6–14.1% (Table 2). The new species shows the lowest K2P value with the *P. quathlambae* 'Eastern' and 'Mohale' lineages (6.6% and 7.6%, respectively) and the highest genetic distance to *P. skeltoni* (14.1%). The *P. quathlambae* 'Eastern' and 'Mohale' lineages are separated by 2.4% genetic distance. The three molecular species delimitation methods (i.e., SP, bPTP, ASAP) identified 15–16 putative species in the Cyt *b* dataset comprising 21 sequences belonging to different nominal species (Fig. 2). All molecular species delimitation methods delimited the *P. kubhekai* sp. nov., *P. quathlambae* 'Mohale', and *P. quathlambae* 'Eastern' lineages as three hypothetical species.

Table 2. Average K2P genetic distance (%) between *Pseudobarbus* spp./lineages.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	*P. kubhekai* sp. nov.																
2	*P. quathlambae* 'Mohale'	7.6															
3	*P. quathlambae* 'Eastern'	6.6	2.4														
4	*P. afer*	12.6	13.4	13.4													
5	*P. swartzi*	12.3	12.8	11.7	4.6												
6	*P. senticeps*	12.1	13.4	12.3	5.3	2.6											
7	*P. afer* 'Forest'	12.6	12.6	11.1	6.6	4.8	4.6										
8	*P. asper*	13.5	14.5	13.6	8.2	6.9	7.4	7.4									
9	*P. tenuis*	10.9	13.4	13.3	6.1	5.3	6.4	8.0	3.6								
10	*P. phlegethon*	13.0	13.7	13.1	4.6	4.3	5.1	4.8	7.7	7.2							
11	*P. burchelli* 'Breede'	10.4	10.9	10.8	6.4	5.6	7	6.7	8.0	7.4	6.7						
12	*P. burchelli* 'Heuningnes'	10.6	11.2	11.0	6.1	5.4	6.7	6.9	7.7	7.2	6.4	1.7					
13	*P. burchelli* 'Tradou'	9.8	10.5	10.3	6.4	6.1	6.9	6.9	8.2	6.9	7.7	4.3	4.6				
14	*P. skeltoni*	14.1	14.6	13.8	9.3	8.2	9.0	7.4	11.2	10.6	9.8	7.7	7.9	7.1			
15	*P. burgi*	10.9	13.9	13.1	6.6	6.1	6.9	6.9	7.7	7.4	6.6	5.8	5.6	6.3	9.3		
16	*P. verloreni*	11	14.3	12.7	7.4	6.6	6.4	7.4	8.5	6.6	8.8	6.4	6.7	6.2	8.8	6.6	

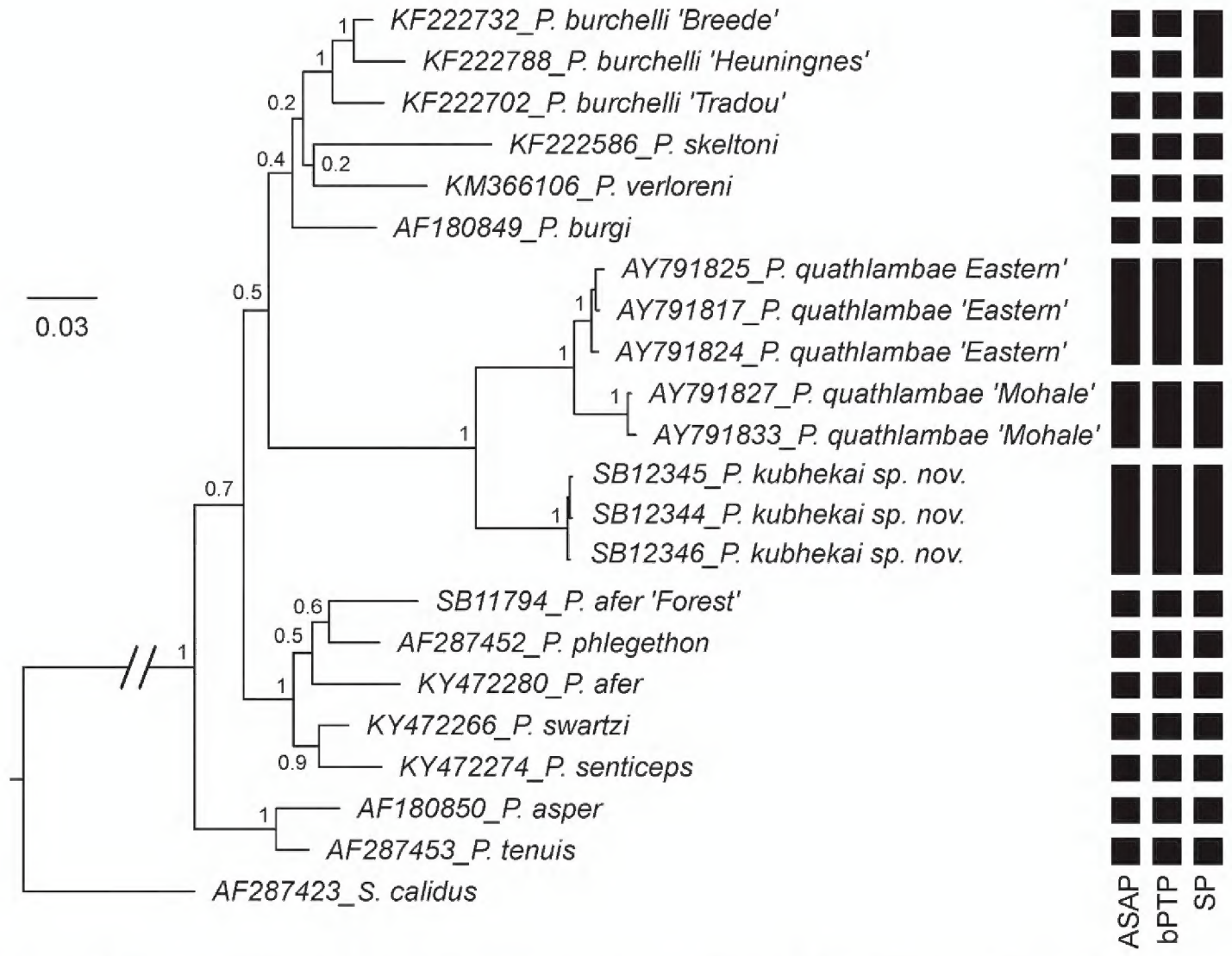

Figure 2. Bayesian phylogeny of *Pseudobarbus* reconstructed based on Cyt *b* sequences, showing phylogenetic placement of *Pseudobarbus kubhekai* sp. nov. Black bars to the right indicate species delimitation results.

Morphological data

The first PCA performed on 10 meristics for 47 specimens of *Pseudobarbus kubhekai* sp. nov. and *P. quathlambae* shows clear separation of the two species based on scale and vertebral counts (Fig. 3A). The first principal component axis (PCI), primarily contrasting differences in the number of scale rows along the lateral line, around the caudal peduncle, and on the predorsal region, explained 91.9% of the total variation (Table 3). The second PCA axis (PCII), primarily contrasting differences in the number of scale rows around the caudal peduncle, only explained 3.5% of the total variation. Specimens of *P. kubhekai* sp. nov. that are situated on the negative part of PCI have fewer scale rows along the lateral line, around the caudal peduncle, and on the predorsal region compared to *P. quathlambae*. Scatterplots of the 10 meristics against standard length showed that *P. kubhekai* sp. nov. and *P. quathlambae* can be clearly separated based on differences in the number of scale rows along the lateral line, scales around the caudal peduncle, scales on the predorsal region, and total vertebrae (Fig. 4). Only a few individuals showed some overlap between the two species in the other meristic characters: lateral line to dorsal fin scales, lateral line to pelvic fin scales, lateral line to anal fin scales, predorsal vertebrae, pre-caudal vertebrae, and caudal vertebrae (Fig. 4). A PCA performed on 17 morphometric characters showed marginal overlapping between *P. kubhekai* sp. nov. and *P. quathlambae* (Fig. 3B). The PCA results for the first four PC axes are shown in Table 4. Scatterplots of the morphometric characters showed considerable overlap for the two species, indicating that these characters are taxonomically uninformative to differentiate these species.

Table 3. Factor loadings for the first two principal component (PC) axes of a PCA carried out on 10 meristic characters (scale and vertebral counts) of *Pseudobarbus kubhekai* sp. nov. and *P. quathlambae*. The most important factor loadings are in bold.

	PCI	PCII
Eigenvalue	70.816	2.693
% Variance	91.88	3.49
Lateral-line scales (LL)	**0.738**	-0.449
Lateral line to dorsal fin scales (LD)	0.068	-0.022
Lateral line to pelvic fin scales (LP)	0.121	0.155
Lateral line to anal fin scales (LA)	0.111	0.152
Caudal peduncle scales (CP)	**0.503**	**0.782**
Predorsal scales (PDS)	**0.392**	-0.311
Total vertebrae (TV)	0.093	0.154
Predorsal vertebrae (PdV)	0.061	0.072
Precaudal vertebrae (PcV)	0.051	0.066
Caudal vertebrae (CV)	0.043	0.094

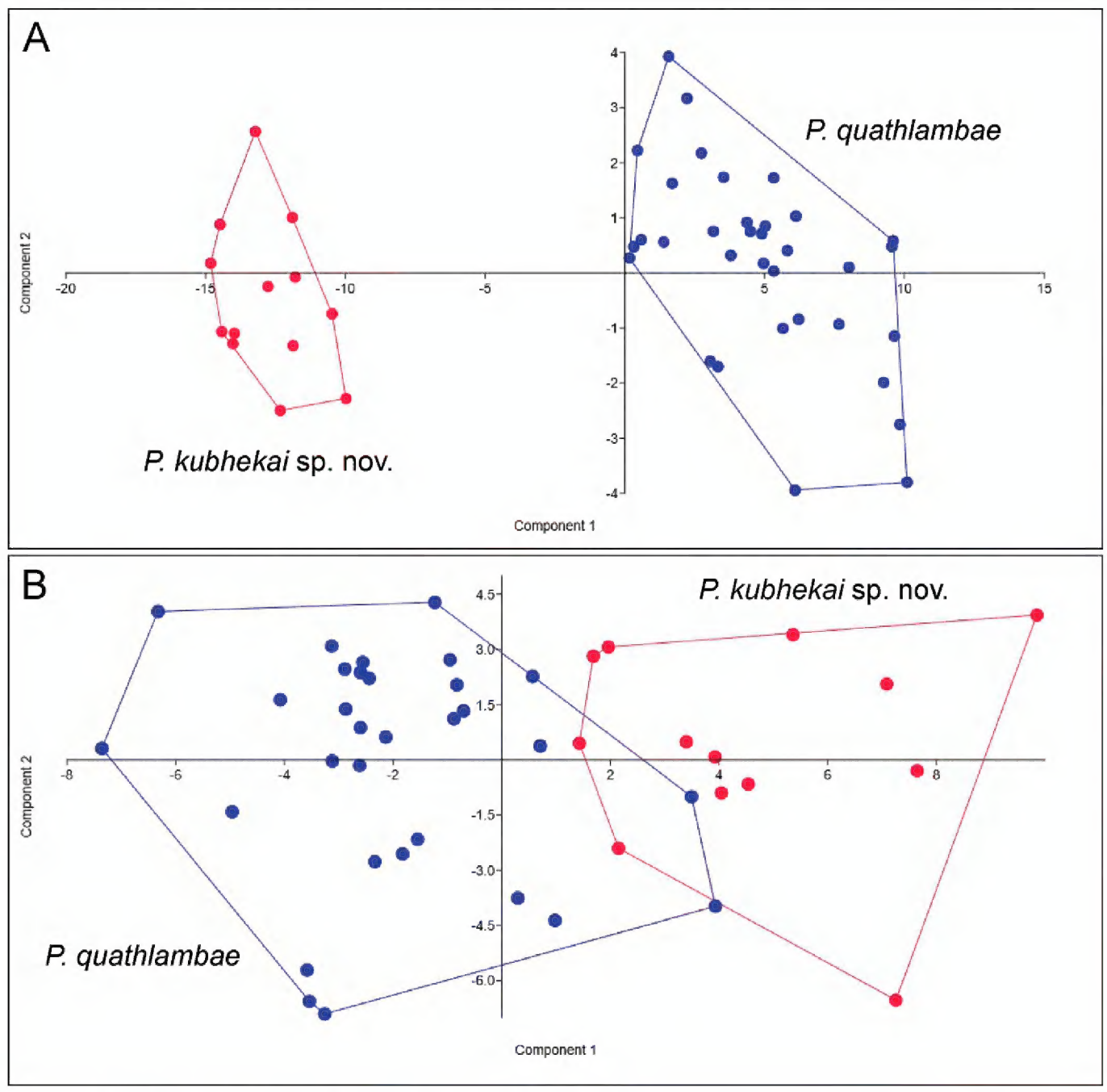

Figure 3. A. Scatter plot of PC1 against PC2 for a PCA carried out on 10 meristic characters (scale and vertebral counts) in *Pseudobarbus kubhekai* sp. nov. (red dots) and *P. quathlambae* (blue dots); **B.** Scatter plot of PC1 against PC2 for a PCA carried out on 17 morphometric characters.

Table 4. Factor loadings for the first four principal component (PC) axes of a PCA carried out on morphometric characters of *Pseudobarbus kubhekai* sp. nov. and *P. quathlambae*. The most important factor loadings are in bold.

	PCI	PCII	PCIII	PCIV
Eigenvalue	15.213	8.994	6.600	4.253
% variance	30.53	18.05	13.25	8.54
% of SL				
Head length (HL)	0.192	-0.224	-0.158	-0.054
Predorsal length (PDL)	-0.167	-0.187	0.268	0.011
Dorsal-fin base (DB)	0.180	-0.013	-0.171	0.006
Dorsal-fin height (DH)	0.212	0.139	-0.280	-0.134
Anal-fin base (AfB)	0.068	0.028	-0.030	-0.010
Pectoral to pelvic fin length (PP)	-0.099	-0.043	**0.429**	0.043
Pelvic to anal fin length (PA)	0.029	0.069	0.174	0.050
Body depth (BD)	0.104	-0.135	0.125	0.263
Body width (BW)	0.034	-0.058	0.175	0.245
Caudal peduncle length (CPL)	-0.069	0.058	-0.133	0.123
% of HL				
Head depth (HD)	-0.019	**0.488**	0.000	0.305
Interorbital width (IO)	0.149	0.364	0.104	**0.574**
Snout length (S)	0.204	-0.146	-0.397	0.389
Postorbital length (PO)	-0.175	0.133	0.082	0.246
Posterior barbel length (PB)	0.102	-0.492	-0.284	0.340
Orbit diameter (OD)	0.222	**0.456**	-0.283	-0.267
% of CPL				
Caudal peduncle depth (CPD)	**0.819**	-0.056	**0.425**	-0.092

Taxonomy

Pseudobarbus kubhekai sp. nov.

https://zoobank.org/F1BB9364-FE1D-43D8-8425-75DF0C38E799

Figs 5, 7

Pseudobarbus quathlambae (non-Barnard)—Kubheka et al. 2017: 303; Swartz et al. 2023: 301.

Proposed common name. Umzimkhulu Redfin (English); Umzimkhulu Rooivlerkie (Afrikaans).

Holotype. SAIAB 204589 (tag number F91), male, 60.5 mm SL, Umzimkhulu River system (exact locality not indicated due to conservation sensitivities), collected by A. Chakona, N. Mazungula, S. Kubheka, and N. Ntuli, 25 May 2017.

Paratypes (n = 12). SAIAB 246079 (tag numbers F85–F90 & F92–F94), 9 unsexed, 45.9–62.9 mm SL, same locality information and collectors as holotype. Paragenetype: SAIAB sequence IDs: SB12344 (tissue ID: AC16FT-264; GenBank number: PQ367261), SB12345 (tissue ID: AC16FT-268; GenBank number: PQ367262), and SB12346 (tissue ID: AC16FT-288; GenBank number: PQ367263). SAIAB 246080 (tag numbers F111–F113), 3 unsexed, 47.2–78.3 mm SL, same locality information as holotype, collected by P.S. Kubheka and N.S. Ntuli, 26 May 2017.

Additional non-type materials (n = 2). SAIAB 246079, 2 unsexed, 39.1–40.6 mm SL, same locality information and collectors as for the holotype.

Diagnosis. *Pseudobarbus kubhekai* sp. nov. is easily distinguishable from *P. burchelli*, *P. burgi*, *P. skeltoni*, and *P. verloreni* by possessing a single pair of oral barbels (vs. two pairs). The new species differs from all currently recognized congeners by having 51–56 scales in lateral-line series (vs. 60–72 in *P. quathlambae* and 25–45 in other species). It further differs from its closest relative, *P. quathlambae*, by having fewer vertebrae (36–37 vs. 38–40) and lacking dark spots on its back (vs. presence of 2–4 rows of dark spots on back; Figs 5–7 for comparison).

Description. All morphometric values in the text are presented as holotypes first and paratypes, if different, in parentheses. The following description is based on holotypes and paratypes from the Umzimkhulu River system.

General morphology. Body proportions and meristics are given in Table 5. Body moderately elongate, fusiform, its depth in front of dorsal-fin origin (deepest) 4.7 (4.4–4.7) in SL, body laterally compressed. Caudal peduncle shallow, its depth half of caudal peduncle length. Head large, length 3.6 (3.6–3.7) in SL, depressed, depth 6.0 (5.7–6.1) in SL and 0.8 (0.7–0.8) of body depth. Dorsal profile of head posterior to orbit steep. Snout blunt, short, oblique, its dorsal profile convex, longer than eye, length 1.6 (1.3–1.6) of eye diameter and 2.9 (2.7–3.0) in head length. Eyes large, diameter 4.7 (4.0–4.7) in head length, and dorsolateral, not extending above dorsal profile, located closer to tip of snout than posterior margin of operculum. Interorbital wide and flat, 1.4 (1.0–1.4) of eye diameter. Mouth sub-terminal, sickle-shaped, its corner reaching vertical through middle of nares. Mouth with a single pair of short maxillary barbels, barbel length 0.6 (0.4–0.7) of orbit diameter, not reaching vertical through middle of eye pupil.

Tuberculation. Snout, lips, barbels, top of head, operculum, preoperculum, suborbital, cheek, and head ventrally covered with numerous minute tubercles in all specimens (Fig. 8). Bands of minute tubercles present on pectoral-fin rays. In specimens larger than 50 mm SL (except two paratypes), larger tubercles present on top of head. In holotype, minute tubercles found on all scales (2–5 tubercles per scale), as well as on dorsal- and pectoral-fin rays.

Scales. Lateral-line scales (LL) 51–56 (holotype: 52; paratypes: 51:5, 52:2, 53:1, 54:2, 55:1, 56:1), LD 9–11 (holotype: 10; paratypes: 9:1, 10:6, 11:5), LP 8–10 (holotype: 8; paratypes: 8:6, 9:5, 10:1), LA 7–9 (holotype: 7; paratypes: 7:4, 8:6, 9:2), CP 22–25 (holotype: 24; paratypes: 22:4, 23:4, 24:3, 25:1), PDS 27–30 (holotype: 29; paratypes: 27:4, 28:2, 29:4, 30:2). Nape, opercle, and cheek naked. Predorsal scales between posterior edge of head and dorsal-fin origin embedded and smaller than flank scales. Triangular naked patch between the gill covers and anterior base of pectoral fins; ventral scales between pectoral-fin base and pelvic-fin origin reduced and embedded. All scales cycloid.

Fins. Dorsal-fin elements iii/7; anal-fin elements iii/5; pectoral-fin elements 15–17 (holotype: 17; paratypes:

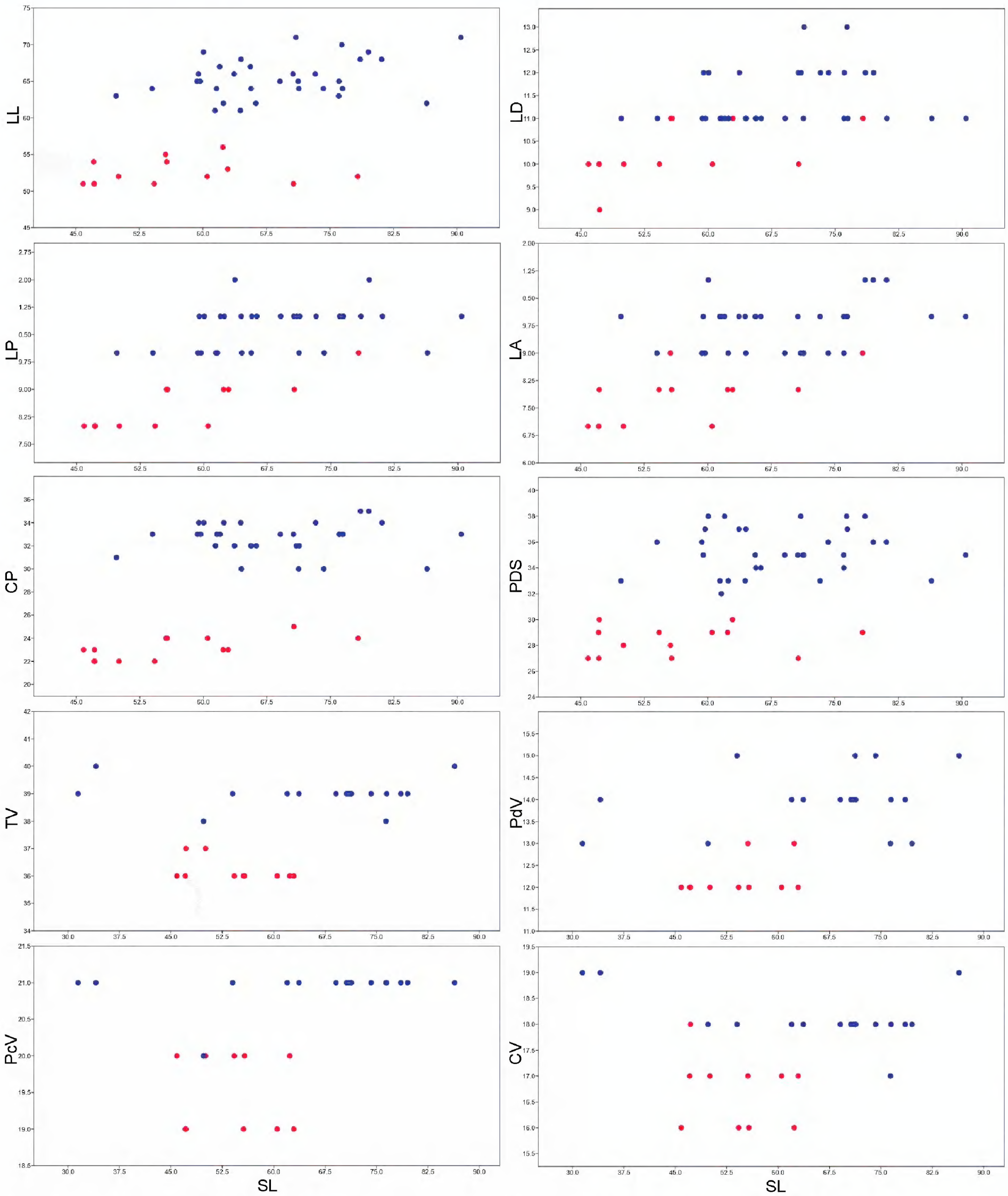

Figure 4. Scatter plots of scale and vertebral counts against standard length (SL) for *Pseudobarbus kubhekai* sp. nov. (red dots) and *P. quathlambae* (blue dots). LL, lateral line scales; LD, lateral line to dorsal fin scales; LP, lateral line to pelvic fin scales; LA, lateral line to anal fin scales; CP, caudal peduncle scales; PDS, predorsal scales; TV, total vertebrae; PdV, predorsal vertebrae; PcV, precaudal vertebrae; CV, caudal vertebrae.

15:1, 16:9; 17:2); pelvic-fin elements 8; caudal-fin principal rays 10+9. Dorsal fin situated almost in the center of the body (including caudal fin), origin slightly behind vertical through origin of pelvic fin, distal margin slightly convex, tip of depressed dorsal fin reaches within 1–3 scales to vertical through posterior base of anal fin. Pectoral fins fan-shaped, length variable, reaches or slightly extending beyond base of pelvic fin in four paratypes, reaches 2–4 scales to base of pelvic fin in other specimens. Pelvic-fin origin slightly in front of dorsal-fin origin, length variable, slightly extending beyond origin of anal fin in four paratypes, reaches 1–2 scales before

Table 5. Morphometric and meristic data for *Pseudobarbus kubhekai* sp. nov. and *P. quathlambae*.

		P. kubhekai sp. nov.			*P. quathlambae*	
		Holotype	Holotype + Other specimens including paratypes (n = 15)		Topotypes + Other specimens (n = 34)	
			Range	Mean ± SD	Range	Mean ± SD
SL	Standard length (mm)	60.5	39.1–78.3	54.5 ± 11.0	31.5–90.4	66.7 ± 12.4
HL	Head length (mm)	16.6	12.7–22.7	15.7 ± 2.9	14.1–22.9	18.1 ± 2.1
% of SL						
HL	Head length	27.5	26.8–28.9	27.6 ± 0.5	24.2–28.9	26.0 ± 1.2
PDL	Predorsal length	52.4	51.9–55.0	53.1 ± 1.0	52.2–59.8	55.0 ± 1.6
DB	Dorsal-fin base	12.1	11.9–13.7	12.6 ± 0.5	9.6–12.3	10.9 ± 0.7
DH	Dorsal-fin height	21.9	20.3–25.2	22.5 ± 1.6	18.8–22.8	20.8 ± 1.1
AfB	Anal-fin base	10.5	9.3–10.9	10.2 ± 0.4	8.2–10.6	9.7 ± 0.5
PP	Pectoral to pelvic fin length	22.3	21.6–25.5	23.1 ± 1.2	21.2–27.5	24.2 ± 1.8
PA	Pelvic to anal fin length	16.3	13.8–16.6	15.3 ± 0.9	12.9–17.8	15.2 ± 1.2
BD	Body depth	21.4	21.4–24.6	22.3 ± 0.8	18.6–24.1	22.0 ± 1.5
BW	Body width	14.7	13.4–16.7	14.8 ± 0.8	13.4–18.1	14.7 ± 1.1
CPL	Caudal peduncle length	24.5	23.2–24.6	24.1 ± 0.6	21.6–26.2	24.1 ± 1.0
% of HL						
HD	Head depth	60.5	59.0–63.8	61.3 ± 1.4	56.6–65.4	61.3 ± 2.2
IO	Interobital width	28.9	26.0–32.3	29.2 ± 1.8	23.8–30.7	27.6 ± 1.9
S	Snout length	34.8	33.0–36.9	35.1 ± 1.2	29.5–35.3	32.3 ± 1.6
PO	Postorbital length	46.6	43.6–47.7	45.8 ± 1.2	44.3–49.9	47.0 ± 1.4
PB	Posterior barbel length	13.7	9.1–16.2	13.3 ± 1.9	7.9–16.6	12.2 ± 2.1
OD	Orbit diameter	21.2	18.4–26.7	23.7 ± 2.5	18.2–24.4	21.2 ± 1.5
% of CPL						
CPD	Caudal peduncle depth	51.5	46.5–56.2	51.2 ± 3.2	40.2–53.4	46.9 ± 2.7
Meristics						
UdR	Unbranched dorsal-fin rays	iii	iii		iii	
BdR	Branched dorsal-fin rays	7	7		7	
UaR	Unbranched anal-fin rays	iii	iii		iii	
BaR	Branched anal-fin rays	5	5		5	
PecR	Pectoral-fin elements		16 (15–17)		16–17 (15–18)	
PelR	Pelvic-fin elements	8	8		8	
LL	Lateral-line scales	52	51–52 (51–56)		65–66 (61–71)	
LD	Lateral line to dorsal fin scale rows	10	10–11 (9–11)		11–12 (11–13)	
LP	Lateral line to pelvic fin scale rows	8	8–10		10–11 (10–12)	
LA	Lateral line to anal fin scale rows	7	7–8 (7–9)		9–10 (9–11)	
CP	Caudal peduncle scale rows	24	22–24 (22–25)		30–34 (30–35)	
PDS	Predorsal scale rows	29	27–29 (27–30)		34–38 (32–38)	
TV	Total vertebrae	36	36 (36–37)*		39 (38–40)**	
PdV	Predorsal vertebrae	12	12 (12–13)*		14–15 (13–15)**	
PcV	Precaudal vertebrae	19	19–20*		21 (20–21)**	
CV	Caudal vertebrae	17	16–17 (16–18)*		18 (17–19)**	

*Counts based on radiographs of the holotype and nine paratypes. **Counts based on radiographs of 18 specimens, including three topotypes.

to origin of anal fin in other specimens. Anal-fin distal margin slightly convex, origin closer to anterior base of pelvic fin than caudal-fin base. Caudal fin forked.

***Osteology* (n = 10).** Vertebral column including Weberian apparatus and urostyle: total vertebrae 36–37 (holotype: 36; paratypes: 36:7, 37:2), predorsal vertebrae 12–13 (holotype: 12; paratypes: 12:7, 13:2), precaudal vertebrae 19–20 (holotype: 19; paratypes: 19:4, 20:5), caudal vertebrae 16–18 (holotype: 17; paratypes: 16:4, 17:4, 18:1).

***Coloration* (fresh specimens).** Refer to Fig. 7 for general live coloration. Dorsal part of the body above the lateral line a uniform olive-brown; body below the lateral line nearly abruptly a pale golden tinged with olive, shading to blemished silvery white and becoming white on the ventral surface. A dark mid-lateral band extending from behind the head to the base of the caudal fin, ending in form of a triangular mark at the base of the caudal fin. Countershading is also clear on the head. The ventral surface of the head is white from below a line extending from above the angle of the mouth around and below the eye and across the operculum. Base of fins bright orange-red. The dorsal and caudal fin rays are pigmented a chocolate brown, darker at their bases and lighter at their outer edges.

***Coloration* (preserved).** Background color in alcohol-preserved specimens pale golden. Countershading

Figure 5. Preserved specimens of *Pseudobarbus kubhekai* sp. nov. from the Umzimkhulu River system. **A.** SAIAB 204589 (tag number F91), holotype, 60.5 mm SL; **B.** SAIAB 246079 (tag number F86), paratype, 62.4 mm SL.

evident on head and body, becoming darker dorsally and lighter ventrally (Fig. 5). Flanks with dark mid-lateral band and a distinct triangular mark at the base of the caudal fin. The bright orange-red pigmentation on base of fins fades in preservative.

Etymology. *Pseudobarbus kubhekai* sp. nov. is named after Skhumbuzo Kubheka from Ezemvelo KZN Wildlife, who, through extensive sampling efforts in search of *Pseudobarbus quathlambae* from its type locality and headwater tributaries of the Umkhomazi and adjacent river systems, discovered this new species from the Umzimkhulu River system. This discovery was significant because it helped to resolve a longstanding debate on the natural occurrence of redfin minnows in the KwaZulu Natal Province of South Africa. The discovery also highlights the conservation significance of the headwater tributaries of rivers draining the Drakensberg Mountain.

Figure 6. Preserved specimens of *Pseudobarbus quathlambae*. **A.** SAIAB 131400 (tag number 84), 70.6 mm SL, Bokong River, Lesotho; **B.** SAIAB 29001 (tag number F99), 79.5 mm SL, Sani River, Lesotho.

Distribution. *Pseudobarbus kubhekai* sp. nov. is currently known from two small streams in the Umzimkhulu River system in the Umzimkhulu Local Municipality in a catchment that is largely underdeveloped, with monoculture tree plantations being the major land use activity (Fig. 1). From a conservation point of view, the precise localities must remain undisclosed because of the restricted geographic range of known populations, until suitable conservation measures for them have been put in place.

Conservation status. Comprehensive surveys to determine the extent of occurrence, population size, conservation status, and effective conservation strategies to ensure the continued existence of the new species within the Umzimkhulu River system are needed. Immediate conservation measures should include securing the newly discovered species and prohibition of the introduction of alien fishes, particularly trout *Salmo trutta* Linnaeus, 1758, *Oncorhynchus mykiss* (Walbaum, 1792) and bass *Micropterus* spp., which are present in many rivers in KwaZulu-Natal.

Figure 7. Fresh specimens of *Pseudobarbus kubhekai* sp. nov. from the Umzimkhulu River system. **A.** SAIAB 246079 (tag number F86), paratype, 62.4 mm SL; **B.** SAIAB 246079 (tag number F90), paratype, 54.3 mm SL.

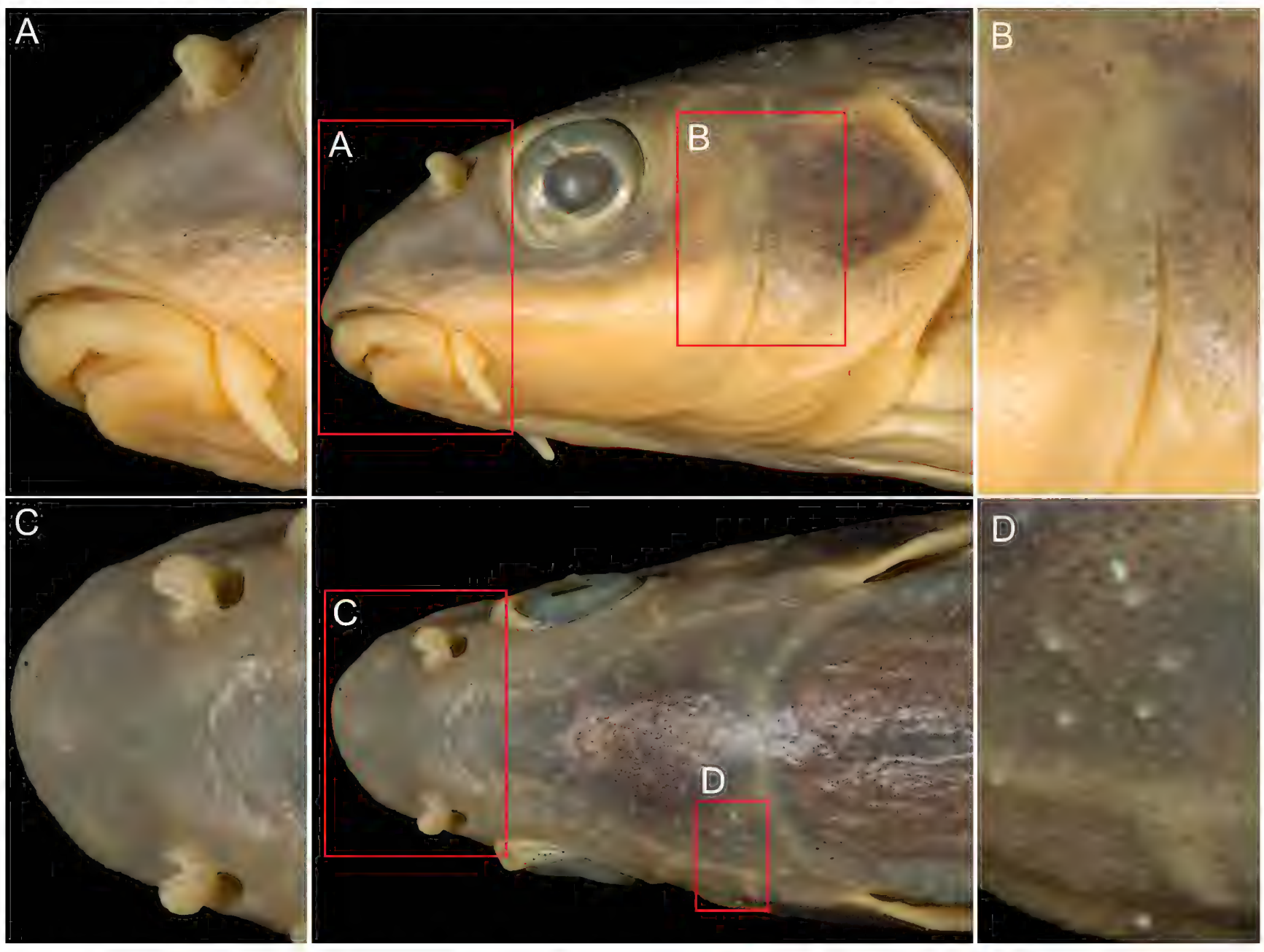

Figure 8. Lateral **(A, B)** and dorsal **(C, D)** surface of head in *Pseudobarbus kubhekai* sp. nov. (SAIAB 204589, holotype, 60.5 mm SL) showing minute and small nuptial tubercle.

Habitat and ecology. The streams in which *Pseudobarbus kubhekai* sp. nov. was found had an average width of approximately 3 m and an average water depth of approximately 0.5 m, with boulders and cobbles as their dominant substratum (Fig. 9). At the time of the surveys, the streams had slow to moderate water flow. The redfins co-occurred with two other cyprinids, Natal yellowfish *Labeobarbus natalensis* (Castelnau, 1861) and bowstripe barb *Enteromius viviparus* (Weber, 1897). Further research is required to further understand the reproduction, ecology, and biology of this taxon.

Discussion

Pseudobarbus, initially erected by Smith (1841) as a subgenus of *Barbus* Daudin 1805, was elevated to generic status by Skelton (1988) to accommodate seven species; however, increased geographic sampling and introduction of genetic data for species identification resulted in a new taxonomic conception of *Pseudobarbus* as a speciose genus comprising 11 currently recognized species (Chakona and Swartz 2013; Chakona et al. 2014; Chakona and Skelton 2017), as well as several putative new species that await formal description (Chakona et al. 2013; Swartz et al. 2004, 2007, 2009, 2023). In the present study, we provided molecular and morphological evidence for distinctiveness of the Umzimkhulu redfin population (Swartz et al. 2023) from all other populations of *P. quathlambae* s.l. and described it as a new species, *Pseudobarbus kubhekai*. Differences between the new species and *P. quathlambae* are provided in the diagnosis.

The discovery of a new population of redfin minnows in the Umzimkhulu River (Kubheka et al. 2017), followed by the determination that this population is genetically distinct from the Lesotho Highlands populations of *Pseudobarbus* (Swartz et al. 2023) and now the confirmation from the present study that the Umzimkhulu River population from KZN represents a distinct species finally resolves the long-standing debate on the natural occurrence of redfins in this region. Swartz et al. (2023) have discussed that river capture events could have potentially played a role in transferring some redfin populations from headwater tributaries of the Orange River system in the Lesotho Highlands to the headwaters of the KZN river systems. This study provides compelling evidence that redfins were previously widely distributed across several upland tributaries of streams draining the Drakensberg Mountain in KZN, but, as argued by Skelton (1988), they were extirpated following the introduction of trout. The recognition of the Umzimkhulu River population from KZN as a distinct species represents a major milestone for the protection of this highly isolated and vulnerable species, as it allows conservation agencies and policymakers to identify and implement appropriate conservation and management strategies to prevent the loss of the remnant populations of this species.

The present study indicates that KZN had at least two species of redfin minnows, *P. quathlambae* s.s. from the Umkhomazi River system and the newly described species, *P. kubhekai*. Considering the geographic separation of the Umkhomazi River system and the headwater tributaries of the Orange River and the genetic separation of redfin populations from eastern and central Lesotho, it is unlikely that the Mohale and Eastern lineages in the Lesotho Highlands are conspecific with *P. quathlambae*. We have, however, refrained from making conclusive decisions in this paper as there is a need for determination of

Figure 9. Type locality of *Pseudobarbus kubhekai* sp. nov. in the Umzimkhulu River system.

the genetic and morphological separation of the Lesotho Highlands lineages from the Umkhomazi specimens. Unfortunately, the Umkhomazi population has gone extinct, and previous and recent efforts have failed to sample and detect redfins from this system. We are exploring the possibilities of generating DNA sequences from the syntypes of *P. quathlambae*, as molecular and detailed morphological and osteological data from these specimens are crucial for resolving the taxonomic status of *P. quathlambae* s.l. (i.e., Umkhomaz and Lesotho Highlands populations). Therefore, we suggest, in the lack of extant material from the Umkhomazi River system, that mtDNA sequences should be recovered from the *P. quathlambae* syntypes, genetically identified and redescribed as *P. quathlambae*, while the remaining lineage(s) not matching genetically the type locality population should be described as new species based on freshly collected specimens from the Lesotho Highlands. In the redescription of *P. quathlambae* and description of the remaining lineage(s), eventual geographic variability of morphology or coloration within each lineage should be checked and described to have description applicable on all populations of these species, e.g., if the type locality population from the Mkhomazana River turned to belong to the Eastern Lesotho lineage, then coloration of the Eastern Lesotho lineage populations should be included in the redescription of *P. quathlambae* to cover the coloration variability of the species. This is the next phase of the ongoing work being undertaken by the NRF-SAIAB research team.

Pseudobarbus kubhekai needs to be listed as a critically endangered species (IUCN 2012) since it is composed of a single evolutionary and management unit (Moritz 1994) with a narrow distributional range and small population size. Rarity and limited distribution associated with a low mtDNA variability can place this species at risk of extinction (Frankham et al. 2002), especially in the face of continuing climatic changes and other extrinsic and ecological factors, such as invasive fish species, deterioration of water quality, impoundments and excessive water abstraction, land use changes, and modification of riverine habitats (Chakona et al. 2022). Apart from the fact that its conservation status, like other species in the genus *Pseudobarbus* (Chakona et al. 2022), has been incompletely assessed, the known genetic, biological, ecological, and distributional data on the Umzimkhulu redfin are also limited. Systematic observations and significant action underpinned by sound scientific evidence are required for taking steps towards its conservation. Immediate measures such as preventing the introduction of alien fishes (e.g., trout, bass, and mosquitofish), identification of suitable habitats based on environmental and ecological niche modeling (Guisan et al. 2017; Whitehead et al. 2017), followed by captive breeding and release of captive-bred individuals into the suitable habitats in the Umzimkhulu system are required for effective conservation of *P. kubhekai*. In addition, not all taxonomic challenges have been resolved; new populations of *P. kubhekai* are likely to be discovered in the KZN, and other redfin populations in the area may undergo taxonomic changes. As noted by Daugherty et al. (1990), good taxonomies 'are not irrelevant abstractions, but the essential foundations of conservation practice'.

Other materials examined for morphology

Pseudobarbus afer (Peters, 1864): SAIAB 34422, 5 males (44.9–65.5 mm SL), 5 females (59.2–74.5 mm SL), Blindekloof River, Groendal Wilderness, Swartkops River system, collected by D. Boulle and P.H. Skelton, 11 November 1988; SAIAB 34428, 5 unsexed (60.1–75.1 mm SL), Blindekloof River, Groendal Wilderness, Swartkops River system, collected by D. Boulle, 8 June 1989; SAIAB121688 (formerly AMG 2524), 24 unsexed (46.0–81.0 mm SL), Elands River, Swartkops River system, -33.7667, 25.1278, collected by P.H. Skelton and A. Bok, 5 September 1974; SAIAB 119909 (formerly AMG745), 5 unsexed (46.0–61.0 mm SL), Elands River, Swartkops River system, -33.71667, 25.1000, collected by R.A. Jubb, 15 February 1964; SAIAB 119773 (formerly AMG 609), 30 unsexed (48.5–66.5 mm SL), Wit River, Sundays River system, -33.3333333, 25.6833333, collected by R.A. Jubb, 8 April 1959; SAIAB 119940 (formerly AMP 776), 5 unsexed (43.0–82.0 mm SL), Kragga Kamma, Baakens River system, -33.9500000, 25.5000000, collected by D. Bicknell, 15 January 1964.

Pseudobarbus afer 'Forest' lineage *sensu* Swartz et al. (2007): SAIAB 237307, 1, male, 67.6 mm SL, Kouma River at Willem's Farm, Klein Brak River system, -33.95261111, 21.97691667, collected by A. Chakona, N. Mazungula, and X. Mathebula, 25 February 2024; SAIAB 246084, 14 unsexed, 46.6–81.7 mm SL, Kouma River at Willem's Farm, Klein Brak River system, -33.95261111, 21.97691667, collected by A. Chakona, N. Mazungula, and X. Mathebula, 25 February 2024; SAIAB 128708, 5 unsexed, 50.1–67.4 mm SL, Causeway at Kruisvallei/George, Keurbooms, -33.812, 23.17472, collected by J. Olivier and S. Thorne, 01 March 1983; SAIAB 200541, 2 unsexed, 74.9–86.9 mm SL, Kwaai River, Keurbooms, -33.82, 23.17972, collected by E. Swartz, 11 April 2000; SAIAB 128186, 3 unsexed, 48.6–52.6 mm SL, Kaapsedrif, Tsitsikamma, -34.16, 24.4, collected by A.H. Bok and M. King, 12 May 1982; SAIAB 64260, 1 unsexed, 68.0 mm SL, Tsitsikamma National Park, Groot River, collected by I.A. Russel, 26 February 2001; SAIAB 122981, 1 unsexed, 47.7 mm SL, Palmietvlei road at Kaapsedrif, Tsitsikamma, -34.05, 24.4, collected by D. Heard, 28 October 1976.

Pseudobarbus phlegethon (Barnard, 1938): SAIAB 51367, 9 unsexed, 51.7–55.7 mm SL, 3–4 km downstream from Algeria, Rondegat River, Olifants System, -32.34999847, 19.0333003998, collected by R. Bills, D. Impson and M. Marriott, 11 March 1996; SAIAB 75826, 5 unsexed, 53.1–60.2 mm SL, Rondegat River, Olifants System, -32.37333297, 19.0602779388, collected by R. Bills, 18 April 2005; SAIAB 75783, 3 unsexed,

49.7–58.6 mm SL, Algeria below weir, Rondegat River, Olifants System, -32.37333297, 19.0602779388, collected by R. Bills, 13 September 2004; SAIAB 58324, 5 unsexed, 54.4–61.8 mm SL, below forestry camp, Rondegat River, Olifants System, -32.35139846, 19.0333003998, collected by R. Bills and D. Naran, 05 February 1998.

Pseudobarbus quathlambae (Barnard, 1938): SAIAB 189215, 3 unsexed (31.5–49.8 mm SL), Himeville, Natal, Mkhomazi River system, South Africa, -29.72078, 29.51226, collected by M. Copeland, unknown date; SAIAB 131399, 7 unsexed (54.0–76.4 mm SL), Jordane River, Lesotho, -29.432, 28.07805, collected by K.J. Meyer, 26 May 1986; SAIAB 25491, 4 unsexed (59.5–76.1 mm SL), Jordane River, Lesotho, -29.39500045, 28.0424995422, collected by K.J. Meyer, 27 October 1985; SAIAB 131400, 4 unsexed (69.1–86.4 mm SL), Bokong River, Lesotho, -29.27027, 28.126, collected by K.J. Meyer, 24 May 1986; SAIAB 29001, 6 unsexed (59.3–79.5 mm SL), Sani River, Lesotho, -29.56083297, 29.2652778625, collected by P.H. Skelton, 22 Septemer 1988; SAIAB 63417, 4 unsexed (61.6–73.3 mm SL), Tsoelikane Falls, Tsoelikana River, Orange System, Lesotho, -29.89749908, 29.1205997467, collected by R. Bills & J. Rall, 02 October 2000; SAIAB 63409, 2 unsexed (76.1–76.5 mm SL), headwaters of Moremoholo River, Orange System, Lesotho, -29.12470054, 29.3271999359, collected by R. Bills & J. Rall, 29 September 2000; SAIAB 63408, 2 unsexed (65.6–65.7 mm SL), Senqu River, Orange System, Lesotho, -28.92280006, 29.0242004395, collected by R. Bills & J. Rall, 29 September 2000; SAIAB 63408, 2 unsexed (81.1–90.4 mm SL), Matsoku River, Orange System, Lesotho, -29.2838993, 28.5531005859, collected by M. Nthimo, 07 February 2000.

Pseudobarbus senticeps (Smith, 1936): SAIAB 304 (holotype), male, 65.7 mm SL, Assegaaibosch River, Krom River system; SAIAB 200302, 9 unsexed, 23–83 mm SL, Assegaaibos River, Krom River system, -33.9452778, 24.3139167, collected by R. Bills, V. Bills, and D. Naran, 12 August 2014; SAIAB 121815 (formerly AMG 2651), 29 unsexed, 45–75 mm SL, Assegaaibosch River, -33.9413889, 24.3188889, Krom River system, collected by P.H. Skelton and J. Stephenson, 20 January 1975.

Pseudobarbus swartzi Chakona & Skelton, 2017: SAIAB 203792 (holotype), male, 80.9 mm SL, Tributary of the Wabooms, Gamtoos River system, -33.8639772, 23.8263333, collected by A. Chakona, B. Motshegoa, N. Mazungula, W. Kadye and R. Smith, 21 January 2015; SAIAB 203793 (Field no: AC15AL39), 9 unsexed, 35.4–76.0 mm SL, Tributary of the Wabooms, Gamtoos River system, -33.8639772, 23.8263333, collected by A. Chakona, B. Motshegoa, N. Mazungula, W. Kadye and R. Smith, 21 January 2015; MRAC 2016-032-P-0001-0004 (Field no: AC16AL02), 4 unsexed, 50.2–61.4 mm SL, main tributary of the Louterwater River, -33.8333611, 23.6373056, Gamtoos River system, collected by A. Chakona, S. Reddy and R. Smith, 18 January 2016; AC16AL01 (SAIAB 203772), 10 specimens, unsexed, 25.5–57.9 mm SL, Western Tributary of the Louterwater River, -33.825750, 23.6310, Gamtoos River system, collected by A. Chakona, S. Reddy and R. Smith, 18 January 2016; AC16AL02 (SAIAB 203779), 6 specimens, unsexed, 32–64.8 mm SL, Main Tributary of the Louterwater River, -33.8333611, 23.6373056, Gamtoos River system, same collectors and date as AC16AL01 (SAIAB 203772); AC16AL04 (SAIAB 203787), 34 specimens, unsexed, 18.2–86.7 mm SL, upper Dwars River, -33.6534444, 23.7539722, Gamtoos River system, same collectors and date as AC16AL01; AC16AL05 (SAIAB 203786), 17 specimens, unsexed, 34.8–64.9 mm SL, Klein River at Kouga Wilderness, -33.7112222, 23.8440833, Gamtoos River system, same collectors as AC16AL01 (SAIAB 203772), 19 January 2016; AC16AL06 (SAIAB 203789), 8 specimens, unsexed, 47.8–70.2 mm SL, Braam River, -33.7135278, 23.8465833, Gamtoos River system, same collectors as AC16AL01 (SAIAB 203772), 19 January 2016; AC16AL07 (SAIAB 203788), 13 specimens, unsexed, 17.9–63.3 mm SL, Diep River, -33.7541944, 24.0812500, Gamtoos River system, A. Chakona and R. Smith, 20 January 2016; AC16AL08 (SAIAB 203781), 45 specimens, unsexed, 14.7–53.8 mm SL, Upper Kansenkei River, -33.7296667, 24.5545833, Gamtoos River system, same date and collectors as AC16AL07 (SAIAB 203788); AC16BL01 (SAIAB 203774), 10 specimens unsexed, 25.5–57.9 mm SL, Wit River, -33.6538333, 24.51605556, Gamtoos River system, A. Chakona and B. Motshegoa, 7 March 2016; AC16BL02 (SAIAB 203780), 5 specimens unsexed, 24.6–58.8 mm SL, Lourie River, -33.8506944, 25.0388194, Gamtoos River system, A. Chakona and B. Motshegoa, 7 March 2016; SAIAB 120538 (formerly AMG1374), 70 unsexed, Kouga Dam, Gamtoos River system, -33.6666667, 24.5166667, collected by F. Farquharson, 6 July 1967; SAIAB 120539, 70 unsexed, same locality and collector as SAIAB 120538.

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References

Barnard KH (1938) Description of a new species of freshwater fish from Natal. Annals of the Natal Museum 8: 525–528.

Chakona A, Skelton PH (2017) A review of the *Pseudobarbus afer* (Peters, 1864) species complex (Teleostei, Cyprinidae) in the eastern Cape Fold Ecoregion of South Africa. ZooKeys 657: 109–140. https://doi.org/10.3897/zookeys.657.11076

Chakona A, Swartz ER (2013) A new redfin species, *Pseudobarbus skeltoni* (Cyprinidae, Teleostei), from the Cape Floristic Region, South Africa. Zootaxa 3686: 565–577. https://doi.org/10.11646/zootaxa.3686.5.5

Chakona A, Swartz ER, Gouws G (2013) Evolutionary drivers of diversification and distribution of a southern temperate stream fish assemblage: testing the role of historical isolation and spatial range expansion. Plos ONE 8(8): e70953. https://doi.org/10.1371/journal.pone.0070953

Chakona A, Swartz ER, Skelton PH (2014) A new species of redfin (Teleostei, Cyprinidae, *Pseudobarbus*) from the Verlorenvlei River system, South Africa. ZooKeys 453: 121–137. https://doi.org/10.3897/zookeys.453.8072

Chakona A, Jordaan MS, Raimondo DC, Bills RI, Skelton PH, van Der Colff D (2022) Diversity, distribution and extinction risk of native freshwater fishes of South Africa. Journal of Fish Biology 100(4): 1044–1061. https://doi.org/10.1111/jfb.15011

Chakrabarty P (2010) Genetypes: a concept to help integrate molecular phylogenetics and taxonomy. Zootaxa 2632: 67–68. https://doi.org/10.11646/zootaxa.2632.1.4

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Molecular Ecology 9: 1657–1659. https://doi.org/10.1046/j.1365-294x.2000.01020.x

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9(8): 772–772. https://doi.org/10.1038/nmeth.2109

Daugherty CH, Cree A, Hay JM, Thompson MB (1990) Neglected taxonomy and continuing extinctions of tuatara (*Sphenodon*). Nature 347(6289): 177–179. https://doi.org/10.1038/347177a0

Frankham R, Ballou JD, Briscoe DA (2002) Introduction to conservation genetics. Cambridge University Press, Cambridge, UK. https://doi.org/10.1017/CBO9780511808999

Guisan A, Thuiller W, Zimmermann NE (2017) Habitat suitability and distribution models: with applications in R. Cambridge University Press, Cambridge, UK. https://doi.org/10.1017/9781139028271

Hall TA (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41(41): 95–98.

Hammer Ø, Harper DAT, Ryan PD (2001) PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4: 9.

Hubbs CL, Lagler KF (1958) Fishes of the Great Lakes Region. Bulletin of the Cranbrook Institute of Science 26: 1–213.

IUCN (2012) IUCN red list categories and criteria: Version 3.1, Second edition. IUCN, Gland, Switzerland and Cambridge, UK.

Jubb RA (1966) *Labeo* (?) *quathlambae*, a rare freshwater fish now feared extinct. Piscator 67: 78–80.

Kubheka PS, Chakona A, Mazungula DN (2017) The Maloti minnow *Pseudobarbus quathlambae* (Barnard, 1938) is not extinct in South Africa. African Journal of Aquatic Science 42(3): 303–306. https://doi.org/10.2989/16085914.2017.1363705

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33: 1870–1874. https://doi.org/10.1093/molbev/msw054

Machordom A, Doadrio I (2001) Evolutionary history and speciation modes in the cyprinid genus *Barbus*. Proceedings of the Royal Society of London. Series B: Biological Sciences 268(1473): 1297–1306. https://doi.org/10.1098/rspb.2001.1654

Moritz C (1994) Defining "evolutionarily significant units" for conservation. Trends in Ecology & Evolution 9(10): 373–375. https://doi.org/10.1016/0169-5347(94)90057-4

Naran D, Skelton PH, Villet MH (2006) Karyology of the redfin minnows, genus *Pseudobarbus* Smith, 1841 (Teleostei: Cyprinidae): one of the evolutionarily tetraploid lineages of South African barbines. African Zoology 41(2): 178–182. https://doi.org/10.1080/15627020.2006.11407353

Pike T, Karssing RJ (1995) Investigation of the presence of Maluti minnows, *Pseudobarbus quathlambae* in the Umkomozana River. Lammergeyer 43: 64.

Pike T, Tedder AJ (1973) Rediscovery of *Oreodaimon quathlambae* (Barnard). Lammergeyer 19: 9–15.

Puillandre N, Brouillet S, Achaz G (2021) ASAP: assemble species by automatic partitioning. Molecular Ecology Resources 21: 609–620. https://doi.org/10.1111/1755-0998.13281

Rambaut A, Drummond AJ (2012) FigTree version 1.4.0. Institute of Evolutionary Biology, University of Edinburgh. http://tree.bio.ed.ac.uk [Accessed on 13 July 2024]

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Sabaj MH (2010) Standard symbolic codes for institutional resource collections in herpetology and ichthyology: An Online Reference. Version 2.0 (8 November 2010). Electronically accessible at http://www.asih.org/, American Society of Ichthyologists and Herpetologists, Washington, DC, 1–43.

Skelton PH (1974) On the life colours and nuptial tubercles of *Oreodaimon quathlambae* (Barnard, 1938) (Pisces, Cyprinidae). Annals of the Cape Provincial Museum (natural History) 9: 215–222.

Skelton PH (1980) Aspects of freshwater fish biogeography in the Eastern Cape. The Eastern Cape Naturalist 24: 16–22.

Skelton PH (1988) A taxonomic revision of the redfin minnows (Pisces, Cyprinidae) from southern Africa. Annals of the Cape Provincial Museum (Natural History) 16: 201–307.

Skelton PH (2000) Flagships and fragments—perspectives on the conservation of freshwater fishes in southern Africa. African Journal of Aquatic Science 25: 37–42. https://doi.org/10.2989/160859100780177929

Skelton PH (2001) A complete guide to the freshwater fishes of southern Africa. Struik Publishers, South Africa, 122 pp.

Smith A (1841) Pisces. In: Illustrations of the zoology of South Africa; consisting chiefly of figures and descriptions of the objects of natural history collected during an expedition into the interior of South Africa in 1834-36. v. 4: 77 unnumb. pp, accompanying Pls. 1–31.

Swartz ER (2005) Phylogeography, phylogenetics and evolution of the redfins (Teleostei, Cyprinidae, Pseudobarbus) in southern Africa. PhD thesis, University of Pretoria, South Africa.

Swartz ER, Skelton PH, Bloomer (2009) Phylogeny and biogeography of the genus *Pseudobarbus* (Cyprinidae): Shedding light on the drainage history of rivers associated with the Cape Floristic Region. Molecular Phylogenetics and Evolution 51: 75–84. https://doi.org/10.1016/j.ympev.2008.10.017

Swartz ER, Flemming AF, Mouton PLF (2004) Contrasting genetic patterns and population histories in three threatened redfin species (Cyprinidae) from the Olifants River system, western South Africa. Journal of Fish Biology 64(5): 1153–1167. https://doi.org/10.1111/j.0022-1112.2004.00375.x

Swartz ER, Skelton PH, Bloomer P (2007) Sea-level changes, river capture and the evolution of populations of the Eastern Cape and fiery redfins (*Pseudobarbus afer* and *Pseudobarbus phlegethon*, Cyprinidae) across multiple river systems in South Africa. Journal of Biogeography 34: 2086–2099. https://doi.org/10.1111/j.1365-2699.2007.01768.x

Swartz ER, Bragança PH, Rall JL, Kubheka SP, Bloomer P, Skelton PH, Chakona A (2023) Conservation of evolutionary patterns and processes in the Maloti minnow, *Pseudobarbus quathlambae* (Cyprinidae, Smiliogastrini), a narrow-range stream fish imperiled by water transfer scheme developments in the Lesotho Highlands. Hydrobiologia 850(2): 301–313. https://doi.org/10.1007/s10750-022-05069-3

Tsigenopoulos CS, Ráb P, Naran D, Berrebi P (2002) Multiple origins of polyploidy in the phylogeny of southern African barbs (Cyprinidae) as inferred from mtDNA markers. Heredity 88(6): 466–473. https://doi.org/10.1038/sj.hdy.6800080

Whitehead AL, Kujala H, Wintle BA (2017) Dealing with cumulative biodiversity impacts in strategic environmental assessment: a new frontier for conservation planning. Conservation Letters 10: 195–204. https://doi.org/10.1111/conl.12260

Xia X (2018) DAMBE7: new and improved tools for data analysis in molecular biology and evolution. Molecular Biology and Evolution 35: 1550–1552. https://doi.org/10.1093/molbev/msy073

Xia X, Xie Z, Salemi M, Chen L, Wang Y (2003) An index of substitution saturation and its application. Molecular Phylogenetics and Evolution 26: 1–7. https://doi.org/10.1016/S1055-7903(02)00326-3

Zhang J, Kapli P, Pavlidis P, Stamatakis A (2013) A general species delimitation method with applications to phylogenetic placements. Bioinformatics 29(22): 2869–2876. https://doi.org/10.1093/bioinformatics/btt499